Shizu KUZUHA*: A new mode of zygospore formation in *Syncephalis* (Mucorales)

葛葉 静*: ハリサシカビ属 (ケカビ目) に於ける新しい接合胞子形成様式

(Pl. VI—VII)

Sexual reproduction in species of *Syncephalis* has received but scant attention, probably because their zygospores are mostly small, hyaline, and inconspicuous. It is in only several, out of about forty known species, that zygospores were reported (van Tieghem, 1875; Bainier, 1882, 1883; Thaxter, 1897; Morini, 1902; Christenberry, 1940; Hunter & Butler, 1975). As to the mode of zygospore formation, two distinctly different types (Cornu-type and Nodosa-type) have been discriminated on the basis of the structure of zygophoric hyphae, and the locus of zygospore origin (Bainier, 1882, 1883; Thaxter, 1897; Benjamin, 1959).

In the course of my study on Japanese *Syncephalis*, zygospores of *S. tenuis* Thaxter and *S. sphaerica* van Tieghem were detected for the first time. Since the mode of zygospore formation in both species is quite dissimilar to either known type and truely new to the genus, it is here described as the third type (Tenuis-type).

Zygospore formation in S. tenuis (Fig. 1-2, Pl. VI) Zygospores of *S. tenuis* were obtained in gross culture of three soil samples from swamps. Sexual reproduction begins with the encounterance of two hyphae possibly of opposite sexual potency. The one, prospective female hypha, gradually increases its thickness from 0.8-2.5 μm to 9-14 μm in diameter, and usually branches a few to several times to construct a dendroid structure composed of robust tubular branchlets. On the contrary, the other, prospective male hypha which is a little finer than the female even in earlier stages, broadens only slightly (up to 1.5-4 μm) and still retains a rather delicate filamentous form. The male hypha, keeping pace with its mate, continues elongation and branching. There-

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* Mycol. Lab., Tokyo Kasei Univ., Kaga, Itabashi-ku, Tokyo 173. 東京家政大学家政学部.

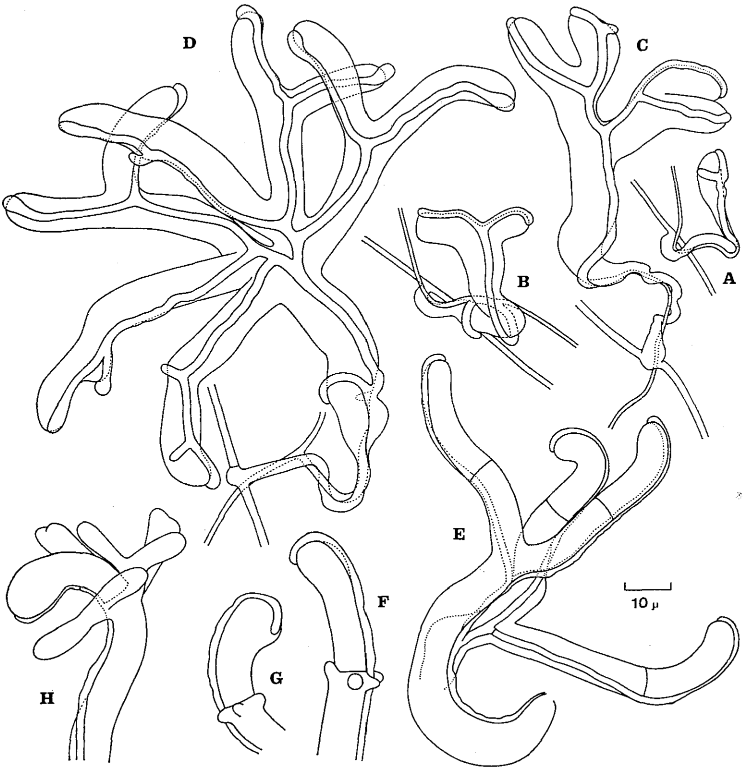

Fig. 1. *Syncephalis tenuis*. A-D. Developing zygophoric hyphae. E. Delimitation of female gametangia. F-H. Enlarging zygospore initial and tubular outgrowths on the female zygophoric hypha.

fore, the tip of each female branchlet is always accompanied by the male counterpart (Fig. 1 A-D, Pl. VI A). Often some peg-like outgrowths are formed from the male hypha, firmly attaching the latter onto the female (Fig. 1 D).

Upon full development, the slightly curved terminal portion of the female branchlet is delimited by a septum as a female gametangium (Fig. 1 E, Pl. VI B). As the male branchlet is considerably fine and filled with the contents, the time of partitioning of the male gametangium was not confidently determined, but probably is soon after the female. Following apical fusion of the paired gametangia, protoplasmic contents of the male gametangium flow into the female one, and the distal portion of the latter begins to swell (Fig. 1.

F-H, Fig. 2 I-K, Pl. VI C-E). The protoplasm is then retracted into this globose portion, which is cut off by a retaining septum as a zygospore (Fig. 2 L, Pl. VI F). Concomitant with the development of zygospore initial, a verticile of a few to several protuberances appears on the female hypha just below the delimiting septum of each gametangium (Fig. 1 F-G). The protuberances mostly

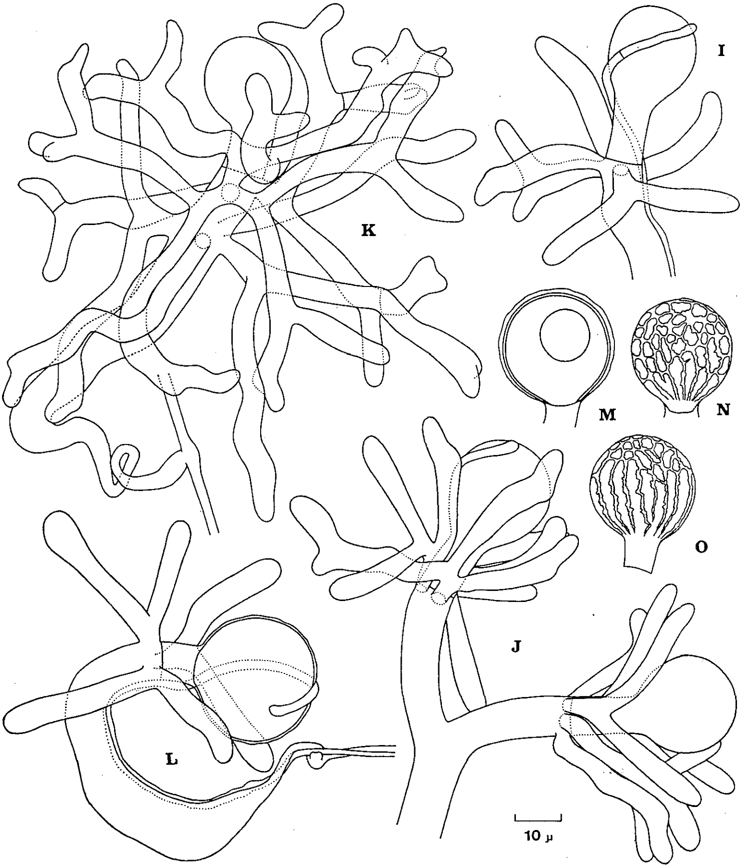

Fig. 2. *Syncephalis tenuis*. I-K. Enlarging zygospore initial(s) and tubular outgrowths on the female zygophoric hypha (K showing somewhat extraordinarily well-developed outgrowths around the zygospore initial. In J and K, the male zygophoric hyphae are partially or wholly omitted.) L. Delimitation of zygospore. M-O. Nearly mature zygospores (M. Optical section. N-O. Surface view.)

branch once or twice during extension and form as a whole a well-developed, dendroid, tubular system encircling the zygospore (Fig. 1 H, Fig. 2 I-L. Pl, VI C-E).

Mature zygospores are globose, hyaline, 20-30 μm in diameter, borne endogenously within the distal swellings of female gametangia and are mostly produced in small groups of up to more than ten on a branched female zygophoric hypha. Exospore (zygosporangium) is derived from the distal portion of the gametangial wall, and at maturity becomes rough with many irregular-shaped thickenings (Fig. 2 M-O, Pl. VI G-I). Endospore wall is smooth. Whether this species is homothallic or heterothallic remains uncertain.

Zygospore formation in S. sphaerica (Fig. 3, Pl. VII) Zygospores of *S. sphaerica* were observed on an American strain which was very kindly offered by Dr. Karen K. Baker as one of her isolates used in the host range study. Her isolate was cultured on a glucose-yeast extract agar[1] together with the following four Mucoralean fungi: *Choanephora cucurbitarum* (Berkeley & Ravenel) Thaxter, *Cokeromyces recurvatus* Poitras, *Mycotypha microspora* Fenner and *Zygorhynchus moelleri* Vuillemin. After about 10 days of incubation at 18°C, zygospore production occurred rather sparingly. Of several media tested, only this medium was effective.

Zygospore formation of this species proceeds in a manner basically similar to *S. tenuis*. Only several minor dissimilarities are recognized between the two. 1) The female zygophoric hyphae of *S. sphaerica* are simple with a single apical zygospore, or often branch once or twice near the base to bear a few zygospores in a smaller cluster. 2) The difference in thickness between the male and the female zygophoric hyphae is in *S. sphaerica* not so distinct as in *S. tenuis*. 3) Outgrowths from the female zygophoric hypha arise not necessarily just below the delimiting septum of the gametangium, but often also from lower portion. They are at first knod-like projections, then become a rather long, bilobed or trilobed, sac-shaped structure, but yet they are shorter and more rounded than those of *S. tenuis*. 4) Mature zygospore are (14-) 17-33 μm in diameter; exospore is rough with relatively large, irregular-shaped, plateaux-like thickenings; endospore wall is smooth and of nearly the same

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[1] 5GY(2) medium: glucose 5 g, yeast extract 5 g, K_2HPO_4 4 g, $MgSO_4 \cdot 7H_2O$ 0.5g, agar 20g, water 1 litter.

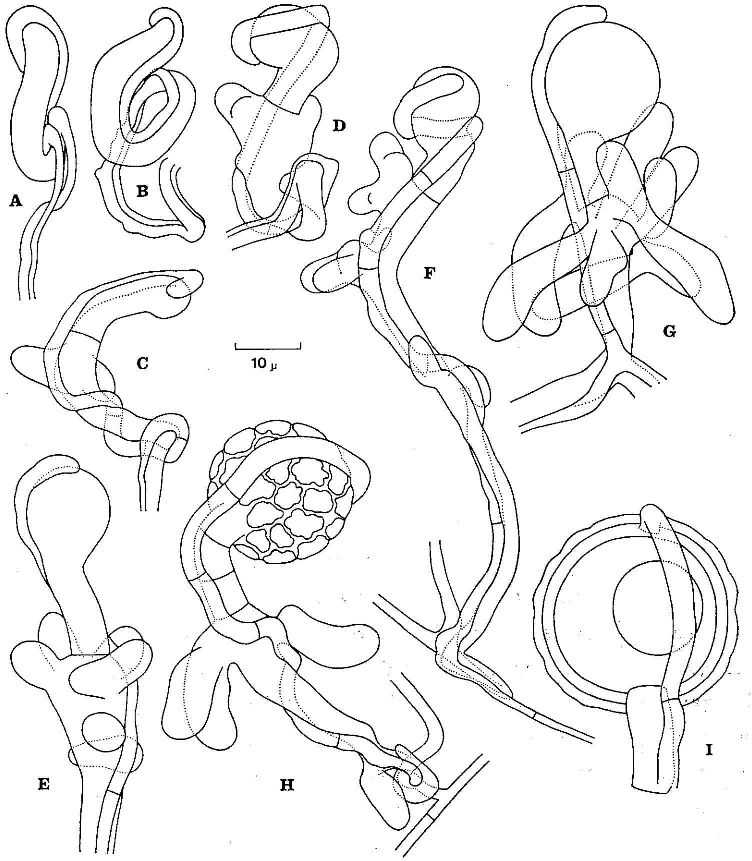

Fig. 3. *Syncephalis sphaerica*. A–B. Developing zygophoric hyphae. C–G. Enlarging zygospore initial and outgrowths on the female zygophoric hypha. H–I. Mature zygospores (H showing surface view. I. Optical section.)

thickness as the exospore, about 2 μm or more. Since these dissimilarities are based on a limited amount of materials, some of them may be a mere reflection of the strain difference rather than the specific one.

In order to determine the sexuality of *S. sphaerica*, some single-spore isolations were made and processed in the same way. But this attempt was unsuccessful, as zygospores could no longer be produced either in single-spore cultures or in their interisolate crossings.

Discussion Sexual reproduction in *Syncephalis* was first detected by van Tieghem (1875) in *S. cornu* van Tieghem & Le Monnier. The zygospore of this species is formed from the point between fused apices of two gametangia which are delimited on apposed progametangia. This type of zygospore formation is tentatively called 'Cornu-type' in the present paper. Some subsequent investigators reported zygospore formation of the Cornu-type in three species: *S. reflexa* van Tieghem (Thaxter, 1897), *S. depressa* van Tieghem & Le Monnier (Christenberry, 1940), and *S. californica* Hunter & Butler (Hunter & Butler, 1975). Zygospore formation of *S. glabra* Morini might be also considered to be of the Cornu-type. However, it is excluded from the discussion here, as Morini's description is so brief and obscure.

The second type of zygospore formation is, up to the present, only known in a single species, *S. nodosa* van Tieghem (Bainier, 1882, 1883; Thaxter, 1897). In the Nodosa-type, the zygospore arises externally on a short stalk from one, somewhat larger, gametangium at a considerable distance from the point of fusion of paired gametangia which are delimited apically on two relatively undifferentiated, spirally twisting zygophoric hyphae (Benjamin, 1959).

The third type, the Tenuis-type, newly described above in *S. tenuis* and *S. sphaerica*, is characterized by the heterogamous conjugation between a larger gametangium delimited on stout, well-differentiated zygophoric hypha and a smaller gametangium on relatively undifferentiated one, and by the endogenous formation of zygospore within the larger gametangium.

Among these three types of zygospore formation, the zygospore of the Cornu-type is constructed under equal participation of two gametangia and no functional difference is notable between the two. On the other hand, in both the Nodosa-type and Tenuis-type, the contents of one, usually smaller, gametangium migrate into the other, usually larger, gametangium immediately after apical conjugation, and the zygospore is formed under more direct participation of the latter. In these cases, accordingly, it may be warrantable to distinguish two gametangia as male and female, respectively. In the Nodosa-type, morphological difference between the male and the female is relatively small and the female gametangium after exogenously producing the zygospore, retains to the last its form as gametangium. While in the Tenuis-type, zygophoric hyphae of both sexes differ so pronouncedly from each other from the early stages of development that the male gives even an impression of a subordinate accompanying organ, and following gametangial conjugation, the female gametangium itself is partially transformed into a zygosporangium, within which a zygospore proper is borne endogenously.

It is of particular interest that the morphological features and reproductive behavior of the female and the male gametangia in *S. tenuis* and *S. sphaerica* bear a close resemblance to those of the oogonium and the antheridium in Oomycetes, especially in species containing a single oospore. We find no parallels in Mucorales, but an analogous form has long been known in the genus *Conidiobolus*, Entomophthorales. When Brefeld (1884) erected this genus basing on *C. utriculosus*, he first observed its sexual reproduction and noted: "so zeigt die Ungleichheit der Anlagen (Anschwellungen) und die Ausbildung der grösseren von ihnen zur Dauerspore, dass wir es mit Formen zu thun haben, welche in ihrer geschlechtlichen Differenzirung über die Zygomyceten hinausgehen und den Oomyceten anzuschliessen sind." But Couch (1939), confirming his observation, concluded that in spite of rather striking affinities with oomycetous fungi shown in external appearance, the resemblance is only superficial and of no phylogenetic significance, for no egg is differentiated in the female structure.

Lastly, what deserves special mention is that in the Nodosa-type and Tenuis-type, vesicular or tubular outgrowths are produced always only from one of paired zygophoric hyphae, the female one. And here, returning to the Cornu-type, in this case also they arise only from one suspensor, not from the both. If it is supposed that these outgrowths are of protective nature and accompany only female zygophoric hypha, their presence can conversely be considered indicative of the female sex. Seen in this light, the presence or absence of vesicular outgrowths on suspensors in the Cornu-type may well be interpreted as reflection of sexual differentiation, or more exactly as retained traces of it. Thus, it may be said that sexual differentiation exists in any type of zygospore formation in *Syncephalis*. van Tieghem early in 1875 applied the words "la différence sexuelle" to the constant difference in length between two conjugating gametangia of *S. cornu*. However, he made no mention of the possible correlation between the outgrowths and "la plus grande cellule femelle".

In the genus *Piptocephalis*, paired gametangia and suspensors are of nearly the same shape apart from a few exceptions, and no such functional difference appropriate to be called sexual is perceptible. The sexual apparatus of the

Tenuis-type in the genus *Syncephalis* can be considered one of the most prominent examples of sexual differentiation throughout the Zygomycetes.

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Explanation of Plates VI-VII

Pl. VI. *Syncephalis tenuis*. A-B. Developing zygophoric hyphae. (B showing apically delimited female gametangia, each accompanied by a male counterpart.) C-D. Enlarging zygospore initials and tubular outgrowths on the female zygophoric hypha. E. Portion of D enlarged. F. Delimited zygospores. G-I. Nearly mature zygospores (G. Optical section. H-I. Surface view.)

Pl. VII. *Syncephalis sphaerica*. A. Zygophoric hyphae at early stage of development. B-D. Enlarging zygospore initials and outgrowths on the female zygophoric hypha. E-H. Mature zygospores (G. Optical section. H. Surface view.) I. Crushed zygospore showing zygosporangial architecture. (Arrows show male zygophoric hyphae or male gametangia.)

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ハリサシカビ属の有性生殖には接合胞子の形成様式により，二つの異なった型が存在することが知られている。その一つ，Cornu-type では，接合胞子は二つの配偶子嚢の対等な関与の下に両者の中間に生じる。一方，Nodosa-type では，接合に引き続いて片方の配偶子嚢の内容物が他方に移入され，接合胞子は後者より外生的に形成される。今回 *S. tenuis* 及び *S. sphaerica* の二種において，これらいずれとも異なる全く新しいタイプの有性生殖様式が見出されたので，第三型 Tenuis-type として報告した。

この型では，二つの配偶子嚢は形態上のみならず機能的にも性格を異にしており，両者の間に雌雄性の分化を考えうるように思われる。Nodosa-type 同様，雄性（小）配偶子嚢の内容物は雌性（大）配偶子嚢内に流入するが，その後，後者の先端部が膨らんで接合胞子嚢へと変形し，内生的に接合胞子を形成する。雌性の zygophoric hypha からは接合胞子を包むように付属突起（枝）も生じ，接合菌類の有性生殖構造としては，最も雌雄性の顕著な例の一つと考えられる。

○クマガイソウの新変種（鈴木昌友） Masatomo SUZUKI: A new variety of *Cypripedium japonicum* Thunb.

クマガイソウは中国（四川），北海道（渡島），本州，四国，九州，また伊豆七島の三宅島まで分布する多年草で，扇形をした二枚の葉には縦じわがあり，茎頂にほゞ対生状に出て，茎や花梗には縮毛が密生し，大形の花をつけるので人目につきやすい。

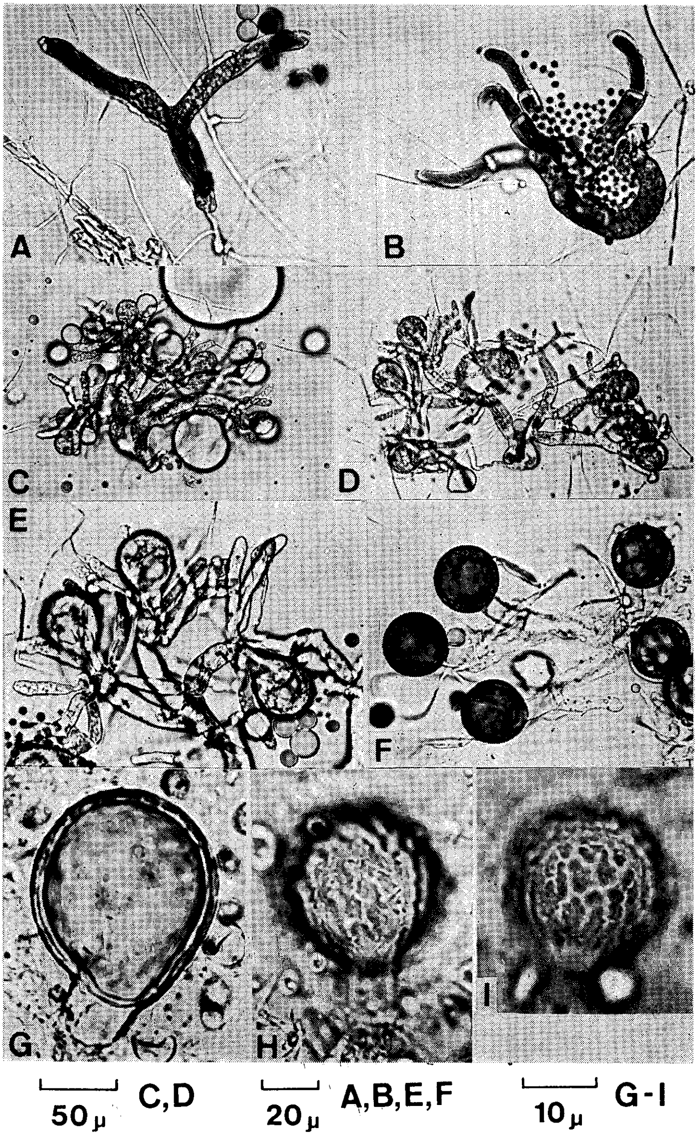
茨城県下では一般に竹籔やスギ林の中などに群生するが，1975年に常北町古内のスギ林下でクマガイソウの集団の中に茎や花梗に縮毛の全く見られない個体が多数生育しているのを見た。縮毛のない個体は以前にも採集した記憶があるので，茨城大学所蔵の標本を調べてみたら，すでに仏頂山，加波山，鶏足山などでも採集されていた。その後，それらの産地に生育する個体や，水戸市その他に移植した個体を数年間観察して来たが，茎や花梗に縮毛のない形質は変らなかった。種子の形成も特に異常はなく，花粉の形態や大きさにも正常のクマガイソウと大差はなかった。茎や花梗に縮毛のない顕著な形質が固定しているので，クマガイソウの変種と考え，下記のような学名を用意し，和名はヒタチ（常陸）クマガイソウと呼ぶことにしたい。

Cypripedium japonicum Thunb. var. **glabrum** M. Suzuki, var. nov.

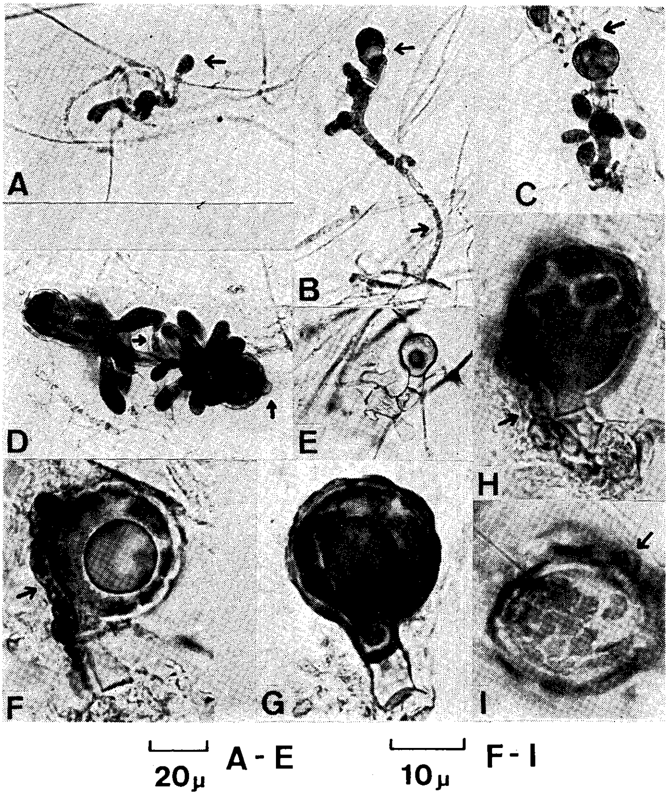
Caulis et pedunculus glaber, cetera ut in typo.

Nom. Jap. Hitachi-Kumagai-so (nov.)

Hab. Japonica. Honshu. Prov. Hitachi: Mt. Bucchô (M. Suzuki, May 3, 1962, typus in Herb. Ibaraki Univ.; M. Yasu, May 8, 1980), Furuuchi, Jyôhoku-machi (M. Suzuki, May 18, 1975), Mt. Keisoku (H. Ogura, May 10, 1969), Mt. Kaba (H.



S. KUZUHA: Zygospore formation in *Syncephalis*



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